

Current Topics

Chemical in Walnuts, Fungal Gene Studies Could Reduce Aflatoxin Risks

When the fungus *Aspergillus flavus* contaminates nuts or other foods, including walnuts, pistachios, almonds, figs, peanuts, corn, and cotton seed, it can produce aflatoxin, a chemical that can cause liver cancer among those who inadvertently consume it. However, efforts to identify a chemical from aflatoxin-blocking walnut varieties and to understand the fungal genes that make aflatoxin or that render such fungi more vulnerable to fungicidal treatments could improve the safety of these foods.

A recent milestone in this quest to reduce the risk from aflatoxin exposures came from identifying a particular walnut variety, named Tulare, that is grown on a commercial scale in California. Unlike other walnut varieties, it is remarkably resistant to producing aflatoxin, according to Bruce Campbell of the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) Plant Mycotoxin Research Unit at the Western Regional Research Center in Albany, Calif., and his collaborators. Tulare walnuts were developed at the University of California, Davis, to expand choices for growth in different climates.

Tulare's resistance depends on its ability to make gallic acid, a potent inhibitor of aflatoxin biosynthesis, Campbell says. Gallic acid is stored in tannins inside the walnut pellicle, the paper-thin coating that surrounds the nut-

meat. The enzyme tannase from *A. flavus* breaks down tannins, releasing gallic acid. Although aflatoxin causes liver cancer in humans and is acutely poisonous to chickens and other types of poultry, it does not appear to hurt the fungus. Moreover, he adds, "aflatoxin is not a chemical required for the fungus to survive ... [and] a number of *Aspergillus* strains [that] don't make aflatoxin ... survive well."

Tulare walnuts generate 1.5 to 2 times more gallic acid than do other walnuts, and those with the least gallic acid tend to be the most susceptible to aflatoxin contamination, according to ARS chemists Noreen Mahoney and Russell Molyneux. Although the high levels of gallic acid in Tulare

walnuts was not bred into them, raising the gallic acid content of other nuts through gene transfer or selective breeding presumably could make them more resistant, Campbell says.

The unusual gallic acid-aflatoxin interaction could help to explain why fungi make toxins at all and, more specifically, why some strains of *Aspergillus* make aflatoxin, Campbell says, suggesting that aflatoxin relieves oxidative stress in fungi. When infected or subjected to drought or high temperatures, plants generate reactive oxygen species. "The fungus fights back by producing aflatoxin to counteract the reactive oxygen species," he asserts. Moreover, fungi that do not make aflatoxin probably have other mechanisms for relieving such stress. Although some researchers argue that



Walnut infected with the aflatoxin-producing fungus *Aspergillus flavus*. (Photo courtesy of Bruce Campbell, USDA-ARS Western Regional Research Center, Albany, Calif.)

aflatoxin serves as an antibiotic to kill other microbes, he notes, that possibility remains unproved.

By using deletion mutants of *Saccharomyces cerevisiae* and an expressed sequence tag library for *A. flavus*, Jong Kim, a molecular biologist at ARS in Albany, learned that gallic acid blocks genes associated with aflatoxin biosynthesis, and *A. flavus* possesses several gene pathways for generating oxidative stress responses. He also finds that genes associated with stress responses are linked to aflatoxin biosynthesis. These results lend support to the idea that “stress responses [are] important to fungal tolerance,” Campbell says.

Applying this genetic information could lead to ways of improving fungicide treatments for walnuts, other nuts, or other foods that tend to become contaminated with aflatoxin. The goal is to destabilize the oxidative stress responses of the fungus, so that it cannot combat an applied fungicide, according to Campbell. “If you can target the antioxidant response systems, you can heighten the fungal susceptibility to commercial fungicides 100- to 1,000-fold,” he says. Available commercial fungicides for nuts are very expensive but not so effective.

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Experts Argue Pros and Cons of Biological Agents Lists, Rules

Federal officials in mid-March issued final biodefense-related rules regulating the possession, use, and transfer of “select” infectious agents and toxins. A few days later, scientists and other experts who spoke during the 2005 ASM Biodefense Research Meeting, held in Baltimore, Md., raised questions about the validity of the lists of microbial agents underlying those rules—in some cases, arguing against

More News on the Flu Virus Front

Recent developments regarding influenza virus include:

- World Health Organization and U.S. and Canadian public health officials scrambled last April to notify clinical testing labs in those two countries and 16 others that they may inadvertently have been sent for proficiency testing purposes samples of a virulent H2N2 influenza virus, a type that circulated in 1957–58. Noting that the overall risk is low, WHO officials are asking labs, including more than 3,500 in the United States, to destroy those samples, fearing that accidental exposures to the virus might lead to cases spreading to individuals born since this virus circulated, who would have little or no immunity to it.
- Officials at the National Institute of Allergy and Infectious Diseases (NIAID) in March began fast-track recruitment of volunteers in three U.S. cities to participate in a clinical trial to evaluate the safety of an experimental vaccine to protect against avian H5N1 influenza virus. Sanofi Pasteur of Swiftwater, Pa., manufactured the trial vaccine, which is an inactivated form of an H5N1 virus isolated last year in Southeast Asia. NIAID also awarded a contract to Chiron Corporation of Emeryville, Calif., to produce a similar H5N1 vaccine for clinical trials.
- Health and Human Services Secretary Michael Leavitt in April announced a \$97-million, 5-year contract to Sanofi-Pasteur to develop an alternative cell-based, rather than the traditional egg-based, approach to producing influenza vaccines. The contract also calls for the company to develop plans for a U.S.-based, cell cultured influenza vaccine manufacturing facility.
- Officials from the Democratic People’s Republic of Korea (North Korea) formally requested assistance in the fight against avian influenza (AI) during an international conference on bird flu, held in Paris, France, last April and sponsored by the World Organisation for Animal Health and the United Nations Food and Agriculture Organisation. Officials from those two organizations also announced the new Worldwide Avian Influenza Network to improve collaborations among reference laboratories specializing in avian and human influenza viruses.

this “tyranny of classifications” and for better, more scientific means for updating and making them into “living documents.”

The select agent list was developed by federal officials working under the auspices of the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., and also with officials from the U.S. Department of Agriculture (USDA) for those agents infecting plants and animals. The list was estab-

lished according to several criteria, including: (i) the effect on human health of exposure to the agent or toxin; (ii) the degree of contagiousness of the agent or potency of the toxin and the methods by which the agent or toxin is transferred to humans; and (iii) the availability and effectiveness of pharmacotherapies and immunizations to treat and prevent any illness resulting from infection by the agent or toxin.

Separately, albeit with considerable